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The digestion of fibre by pigs

2. Volatile fatty acid concentrations in large intestine digesta

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1. The effect of including lupin (*Lupinus* sp.) hulls, maize cobs, wheat bran and lucerne (*Medicago sativa*) stems in a basal fibre-free diet on the concentrations and the relative proportions of volatile fatty acids (VFA) in the proximal colon of pigs, 17–18 h after feeding, was studied.

2. Concentrations of total VFA in the proximal colon increased with increasing levels of neutral-detergent fibre (NDF) intake, and this increase was highly dependent on the source of NDF in the diet.

3. Molar proportions of the VFA were significantly affected by the level of NDF intake only in the cases of acetic and butyric acids, whereas the source of dietary NDF had a marked influence on the molar proportions of all acids.

4. The results indicate that the extent of fermentative breakdown of fibre in the pig intestine can be influenced substantially by the type and the level of fibre in the diet.

That animals are capable of digesting considerable amounts of dietary fibre is well established. In sheep, ox and deer, microbial breakdown of cell-wall polysaccharides and subsequent fermentation of the end-products occurs mainly in the rumen and reticulum; in rats and rabbits it occurs in the caecum and the first portion of the colon (Elsden *et al.* 1946; Cranwell, 1968). In all cases, fermentation results in the production of a mixture of volatile fatty acids (VFA), mainly consisting of acetic, propionic and butyric acids. The relative concentrations of these acids in the alimentary tract of several species of animals, including sheep, ox, red deer, horse, rabbits, rats and pigs, were reported to be very similar in all species, except rats, and were on average 67% acetic, 19% propionic and 14% butyric (Elsden *et al.* 1946). Similar concentrations and relative proportions of VFA in the contents of the caecum and the colon of pigs have been reported by Friend *et al.* (1963), Argenzio & Southworth (1975) and Clemens *et al.* (1975).

While, in ruminant animals, the concentrations and the relative proportions of the VFA in the rumen can be markedly influenced by the type of diet (Blaxter, 1962), this aspect has not been examined in detail with pigs. In earlier studies (Friend *et al.* 1962, 1963), it was claimed that the concentrations of VFA in the faeces and in the contents of the large intestine were influenced by the inclusion of cellulose in a standard pig ration. However, recently Gargallo & Zimmerman (1980) were unable to observe any significant changes in the concentrations of VFA in the caecal contents of pigs given diets containing low or high levels of cellulose. These conflicting results may be due, in part, to the source of cellulose which, in its purified form, differs considerably from natural fibres and hence it might be expected to respond differently to microbial digestion (Van Soest, 1978). It appears, therefore, that more information is needed concerning the effect of different types of natural fibre on the production of VFA in the lower parts of the alimentary tract of pigs fed on high-fibre diets.

The objective of the present study was to obtain information as to whether the level and

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the type of dietary fibre would result in any differences in the concentrations and the relative proportions of VFA in the proximal colon of growing pigs. The efficiency of VFA recovery by a procedure in which steam-distillation of a centrifuged sample of digesta was deleted was also explored.

MATERIALS AND METHODS

Animals and diets

The values for the present study were obtained from pigs given 856 g daily of a basal, non-fibre diet including casein, starch, sucrose and maize oil and four levels of lupin (*Lupinus* sp.) hulls, wheat bran, maize cobs and lucerne (*Medicago sativa*) stems that gave rise to diets (treatments) containing 75, 150, 225 and 300 g neutral-detergent fibre (NDF)/kg dry matter (DM) and daily intakes by the pigs of 69, 151, 249 or 367 g NDF. Feed was given in the form of gruel in two equal meals at 08.00 and 16.00 hours. There were three pigs per treatment, a total of forty-eight pigs, of the Large White breed and of an initial mean body-weight of 45 (SE 2) kg. The animals were maintained in metabolism cages and were given the experimental diets for 30 d. Detailed descriptions of pig feeding and management, diet composition and chemical analysis of diets and fibre sources has been made by Stanogias & Pearce (1985).

Sampling procedure

At the end of each digestion experiment the pigs were taken to a slaughter-house at around 09.00 hours, about 17–18 h after they were last fed, which was the time that would allow minimal holding of the pigs in the yards before slaughter and, hence, minimal ingestion of foreign material. The pigs were processed according to a routine slaughter-house procedure and their digestive tracts removed for further examination. Samples for VFA determination were obtained within 0·5–1 h after slaughter. A segment of the proximal colon starting immediately after the junction of the caecum with the colon and extending for about 100 mm was separated by ligature and its total contents were then collected for determination of DM and VFA concentrations. It was not possible to establish the extent to which mixing of the contents of the caecum and colon may have occurred between the moment of slaughter and the time of sampling.

The proximal colon was selected as a sampling site for VFA determinations because it has been reported (Argenzio & Southworth, 1975) that the highest concentration of VFA occurs later than in the caecum. Known portions of the colonic contents were transferred to jars containing 1 M-sulphuric acid saturated with $MgSO_4 . 7H_2O$. The jars were stored overnight at room temperature and then centrifuged at 16000 ***g*** to obtain a clear supernatant fraction. The DM of the contents was determined by drying samples to constant weight in a forced-draught oven at 105°.

Determination of VFA

Total VFA were estimated by titrating two 100 ml distillates of 5 ml supernatant fraction with 0·02 M-sodium hydroxide under carbon dioxide-free conditions using phenolphthalein as indicator. After titrating, the distillates were made alkaline by adding 2–2·5 ml 0·1 M-NaOH, reduced in volume over a steam-bath, transferred into a 15 ml glass vial and finally taken to dryness in a forced-draught oven at 85°.

Samples of the VFA salts were analysed for molar proportions of VFA in a Hewlett-Packard Model 402 gas–liquid chromatograph equipped with flame-ionization detectors and a glass column (12 mm i.d.) packed with acid-washed Chromosorb. Orthophosphoric acid (850 ml/l; 100 μl) was added to the dried sample to free the acids from their sodium salts. The sample was then dissolved in 2·4 ml distilled water, the sodium phosphate salts allowed to settle and 1 μl of the supernatant solution was injected into the column. The same

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amount of supernatant fraction of the acidified and centrifuged samples, taken from the colonic contents of the pigs given maize cobs, that had not been subjected to steam-distillation was also injected into the column. Operating criteria for the gas–liquid chromatograph were as follows: the temperatures for the injection port, the flame-ionization detector and the oven were 225, 225 and 190° respectively. The carrier gas was nitrogen, with a flow-rate of 50 ml/min. Hydrogen and air flow to the detector were 30 and 150 ml/min respectively.

Standard solutions containing reagent grade acetic, propionic, butyric, isobutyric, valeric, isovaleric and caproic acids in various proportions were also prepared for gas–liquid chromatography in the same way as described previously and the widths of the half peak-heights and the peak-heights of these solutions were used to calculate the unknown concentrations of VFA in the samples. This method of steam-distillation followed by gas–liquid chromatography gave recoveries of 97% for a standard mixture of VFA.

Statistical analysis

The values were analysed statistically by single classification analysis of variance or as a 4 × 4 factorial design. The two factors were the source of fibre in the diet (FS) and the level of NDF in the diet, with orthogonal (NDF), linear (NDF_L) and quadratic (NDF_Q) effects. The interaction sum of squares (FS × NDF) was split into linear (FS × NDF_L) and quadratic (FS × NDF_Q) components. These statistical procedures were used as described by Snedecor & Cochran (1973).

RESULTS

The mean values of the concentrations of total VFA in the colonic contents of pigs given different types of fibre at four levels of daily intake are shown in Table 1. The main effect on the concentrations of VFA in the proximal colon of the level of NDF intake by the pigs was the linear increase in VFA concentration with the level of NDF in the diet ($P < 0.001$). There was no evidence of curvilinearity. However, VFA concentrations differed markedly among groups of pigs given diets containing NDF from different sources ($P < 0.001$) and significant interactions between the source of NDF and both the linear ($P < 0.001$) and the quadratic ($P < 0.05$) components of NDF level were found.

The effects of the level and the source of dietary fibre on the molar proportions of individual VFA in the digesta were variable. As can be seen in Table 2 the proportion of acetic acid was significantly ($P < 0.001$) influenced by both the level and the source of NDF in the diet, the effect of the level of NDF being a linear reduction ($P < 0.01$) in the proportion of this acid with increasing NDF intakes, although there was some evidence of curvilinearity ($P < 0.05$). Significant interactions between the source and both the linear ($P < 0.001$) and the quadratic ($P < 0.01$) components of the NDF level were found implying that the changes in acetic acid concentration with the level of NDF intake were highly related to the source of NDF in the diet.

The proportion of propionic acid was significantly affected ($P < 0.01$) by the source of NDF in the diet but not by the level of NDF intake, although a significant interaction between the linear component ($P < 0.05$) of the NDF level and the NDF source was found. The proportion of butyric acid increased curvilinearly with significant linear ($P < 0.01$) and quadratic ($P < 0.01$) effects as NDF intake increased. The means of these increases were significantly different ($P < 0.001$) among groups of pigs receiving diets containing NDF from different sources.

Changes in the molar proportion of isobutyric acid in the colonic contents appeared to be related only to the source of NDF in the diet ($P < 0.001$).

In Table 3 are shown means with their standard errors for the concentration of the total

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Table 1. *Concentration of total volatile fatty acids (VFA; mmol/g dry matter (DM)) in contents of the proximal colon of pigs given different fibres at four levels*

(Mean values with their standard error for sixteen groups of three pigs each)

Source of fibre	Dietary NDF levels (g/kg DM) 75	150	225	300	SEM	FS	NDF† L	NDF† Q	FS × NDF L	FS × NDF Q
Lupin (*Lupinus* sp.) hulls	0·266	0·265	0·308	0·273	0·0272	***	***	NS	***	*
Wheat bran	0·181	0·356	0·432	0·458						
Maize cobs	0·139	0·142	0·213	0·253						
Lucerne (*Medicago sativa*) stems	0·233	0·281	0·311	0·308						

Statistical significance of effects: SEM, FS, NDF†, FS × NDF.

FS, main effect of fibre source; NDF, neutral-detergent fibre; L, linear; Q, quadratic; NS, not significant.
* $P < 0·05$, *** $P < 0·001$.
† Main effect of level of NDF in diet.

Table 2. *Proportions of volatile fatty acids (VFA) in contents of the proximal colon of pigs given different fibres at four levels*

(Mean values with their standard errors for sixteen groups of three pigs each)

VFA	Source of fibre	Dietary NDF level (g/kg DM) 75	150	225	300	SEM	FS	NDF† L	NDF† Q	FS × NDF L	FS × NDF Q
Acetic acid	Lupin (*Lupinus* sp.) hulls	0·726	0·744	0·703	0·730	0·013	***	**	*	***	**
	Wheat bran	0·684	0·690	0·579	0·610						
	Maize cobs	0·738	0·738	0·729	0·730						
	Lucerne (*Medicago sativa*) stems	0·775	0·712	0·736	0·765						
Propionic acid	Lupin hulls	0·194	0·159	0·186	0·181	0·012	**	NS	NS	*	NS
	Wheat bran	0·174	0·154	0·251	0·211						
	Maize cobs	0·202	0·177	0·208	0·208						
	Lucerne stems	0·169	0·203	0·178	0·173						
Butyric acid	Lupin hulls	0·070	0·091	0·105	0·084	0·009	***	**	**	NS	NS
	Wheat bran	0·119	0·151	0·166	0·175						
	Maize cobs	0·047	0·047	0·055	0·052						
	Lucerne stems	0·051	0·077	0·083	0·059						
Isobutyric acid	Lupin hulls	0·010	0·006	0·006	0·004	0·003	***	NS	NS	NS	NS
	Wheat bran	0·003	0·005	0·004	0·004						
	Maize cobs	0·013	0·018	0·008	0·010						
	Lucerne stems	0·005	0·008	0·003	0·003						

Statistical significance of effects: SEM, FS, NDF†, FS × NDF.

FS, main effect of fibre source; NDF, neutral-detergent fibre; DM, dry matter; L, linear; Q, quadratic; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Main effect of level of NDF in diet.

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Table 3. *Concentration of total volatile fatty acids (VFA) (mmol/g dry matter (DM)) measured by different procedures in the contents of the proximal colon of pigs given maize cobs at four levels*

(Mean values with their standard errors for three pigs)

Dietary neutral-detergent fibre level (g/kg DM)	Analytical procedure						Statistical significance of difference
	Titration only		Gas–liquid chromatography after titration		Gas–liquid chromatography without titration		
	Mean	SEM	Mean	SEM	Mean	SEM	
75	0·139	0·0198	0·146	0·0271	0·131	0·0160	NS
150	0·142	0·0435	0·143	0·0418	0·163	0·0532	NS
225	0·213	0·0181	0·230	0·0167	0·233	0·0114	NS
300	0·253	0·0422	0·279	0·0375	0·262	0·0483	NS
Pooled values	0·187	0·0203	0·200	0·0222	0·197	0·0224	NS

NS, not significant.

VFA in the colonic contents of the pigs given maize cobs as determined by distillation and titration or by gas–liquid chromatography of distilled and undistilled samples from the colonic contents of these pigs.

There were no significant ($P > 0·05$) differences due to the method of determination of the total VFA either among the means from each level of NDF intake or among the pooled means.

DISCUSSION

The role of the level and the type of dietary fibre as factors influencing the production, concentrations and proportions of VFA in the large intestine of simple-stomached animals has been a controversial one. For instance, it has been reported that increasing the level of cellulose in the diet may result in a decreased production of VFA in the large intestines of pigs (Friend *et al.* 1963; Argenzio & Southworth, 1975; Imoto & Namioka, 1978) or in an increased concentration of VFA in the contents of the caecum of rats (Yang *et al.* 1969), or it may have no effect on either the concentration or the molar proportion of VFA in the large intestine of pigs (Farrell & Johnson, 1972; Gargallo & Zimmerman, 1980). One possible explanation for these variable responses might have been the source and hence the extent and rate of fermentability of cellulose used in each study.

The results presented here show that the concentrations of VFA in the contents of the upper part of the colon of pigs given different types of fibre can vary with the type of fibre fed. The differences, however, in the concentrations of VFA, although statistically significant, were not sufficiently large to illustrate clearly the effect of the type of fibre.

Argenzio & Southworth (1975) have shown that VFA are readily absorbed by the intestinal mucosa and that the highest concentrations of VFA in the contents of the caecum and the proximal section of the large intestine were reached 2–4 h after feeding. Similarly, Ludvigsen & Thorbek (1961) suggested that the concentration of VFA in the caecal contents of pigs given lucerne may be higher within a few hours after the morning meal. In the present study, the samples from the contents of the upper colon of the pigs were taken 17–18 h after the pigs were last fed. It is possible, therefore, that at the time of sampling of the colon contents the concentrations of VFA were not at their maxima. Had the samples been taken

at the time when VFA production was at its maximum, the effect of the type of dietary fibre on VFA production might have been illustrated more distinctly. In addition, no allowance could be made here for the effects of the possible mixing of caecal and colonic contents between the times of slaughter and sampling.

Nevertheless, the obvious trend towards increased concentrations of VFA with increasing consumption by the pigs of NDF might suggest that greater quantities of fermentable substrate were made available to the micro-organisms in the caeco-colonic region of the digestive tract. If these increased quantities of VFA were absorbed by the intestines and metabolized into energy then, presumably, more energy would be made available to the pigs with higher intakes of dietary NDF.

With the exception of wheat bran, the mean molar proportions of the VFA in the colonic contents of the pigs receiving all other fibres were very similar and typical of those found in animals that possess caeca or large intestines capable of bacterial fermentation such as guinea-pigs, rabbits, horses and pigs, with ruminants included. The reason for the lower proportion of acetic acid and the higher proportion of butyric acid in the colonic contents of the pigs given wheat bran, compared with the pigs given the other fibres, is not clear, but the effect of small amounts of aleurone and endosperm starchy cells attached to the bran flakes, that might have escaped digestion in the stomach and the small intestine, cannot be excluded. Mason & Just (1976) found that the ingestion by pigs of potato starch, which is known to accumulate in the large intestine where it is digested by bacteria (Baker *et al.* 1950), resulted in a higher proportion of butyric acid in the total VFA than when maize starch, which is almost completely hydrolysed in the small intestine, was included in the diets of pigs. It is not clear from the present results whether or to what extent fermentability of a particular type of fibre may be a factor influencing the molar proportions of VFA in the contents of the colon of pigs given different types of fibre.

The results obtained when samples of colonic contents from pigs given maize cobs were subjected or not to steam-distillation, before injection in the gas–liquid chromatograph, confirm earlier reports (Erwin *et al.* 1961) and suggest that valuable time can be saved by avoiding the intermediate steps of steam-distillation, formation of sodium salts and evaporation, in the preparation of such biological samples for gas–liquid chromatographic analysis.

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